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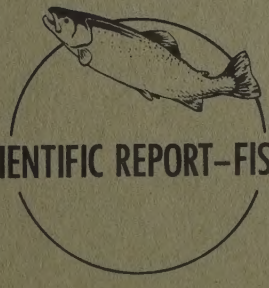
METHODS OF INFECTING TROUT WITH KIDNEY DISEASE AND SOME EFFECTS OF TEMPERATURE ON EXPERIMENTAL INFECTIONS



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METHODS OF INFECTING TROUT WITH KIDNEY DISEASE AND SOME EFFECTS OF TEMPERATURE ON EXPERIMENTAL INFECTIONS

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ABSTRACT

Transmission of kidney disease was studied in eastern brook trout (Salvelinus fontinalis) held at 12.5° C. Fish were subjected to almost daily inoculations or exposures for 102 days, and observations were continued for an additional 200 days. Two strains of the causative bacterium were compared. The bacterium was fed alone, with glass splinters, and with bile salts. It was also inoculated by two methods of abrasion. Other fish were inoculated subdermally and held at 7° C. and at 12.5° C. in order to determine the effects of these two temperatures on the kidney disease infections.

Infection was not successful by addition of the bacterium alone to the diet or with glass shards or with bile salts. Infection was accomplished by manual abrasion and autoabrasion in the presence of the bacterium. The eastern strain of the bacterium infected fish more readily than the western strain; all infections developed slowly and were chronic.

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METHODS OF INFECTING TROUT WITH KIDNEY DISEASE AND SOME EFFECTS OF TEMPERATURE ON EXPERIMENTAL INFECTIONS

In the hatchery or rearing facility devoted to salmonid production, infectious diseases often constitute a serious problem. Kidney disease is one such infection; it is chronic bacteremia which results in appreciable losses among the commercially important Pacific salmon and among the three most important species of hatchery-propagated trout. The cause of the disease has been clearly established. Although the causative bacterium is not yet completely described, it is considered by Ordal and Earp (1956) to be a Corynebacterium.

The loss of these fish can be reduced or eliminated by three general approaches: (1) breeding disease-resistant stocks; (2) preventing the disease; and (3) treating infected fishes when the disease does occur.

Rational prevention of kidney disease presupposes the knowledge of how the disease is transmitted. Unfortunately, the means by which kidney disease is transmitted is as yet unknown. The second approach to the reduction of loss from kidney disease, that of therapy, is severely limited because a satisfactory treatment of the disease has not been found.

The route by which an organism invades or gains entrance to a host is termed its portal of entry. Infectious diseases usually involve one principal portal of entry. In fact, some infections can occur only through a very particular portal of entry. Knowledge of how a disease of fishes can be established under artificial conditions might well indicate the probably portal or portals of entry under hatchery conditions.

Thus far, trout can be experimentally infected with kidney disease only by injection. This method is severe and highly artificial. It is desirable to have a less drastic method whereby nearly natural infections with kidney disease can be conveniently established among large numbers of trout.

Among genera, species, and even among strains of a particular species of fish, differential susceptibility to a particular disease is well known. Kidney disease is generally conceded to be more severe among Pacific salmon than among trout. Eastern brook trout (Salvelinus fontinalis) are more severely affected than rainbow trout Salmo gairdneri). Nothing is known of the range of virulence of the pathogen.

As indicated by Snieszko (1954) in a recent review of therapy of bacterial diseases of fishes, temperature has been found to strongly influence the course of such infections. There is evidence that kidney disease is similarly affected.

The objectives of the research reported here, therefore, were as follows: (1) to attempt to uniformly infect large numbers of trout with kidney disease by different methods and with two different strains of the organism; (2) to observe the course of infections and comparative success achieved by the different methods and by the different strains of the organism; and (3) to determine the effects, if any, of different temperatures on the course of the kidney disease infection.

PORTALS OF ENTRY IN KIDNEY DISEASE INFECTIONS

Ulcer disease and furunculosis, two common bacterial diseases of trout, are routinely transmitted to susceptible hosts by a single day's feeding or by short-term contact with the causative organisms. There is adequate evidence that the same portals of entry are involved in natural and hatchery occurrences of these diseases. Experimental infection with kidney disease has not followed this pattern, and the observations which have been made do not constitute a firm basis for postulating the portal or portals of entry of natural infections.

Earp et al. (1953) reported successful transmission to fingerling blueback salmon (Oncorhynchus nerka) by two or more feedings of infected tissues, but the disease also occurred among control fish. Wood and Wallis (1955) fed infected viscera to fingerling chinook salmon (O. tshawytscha) for 41 to 52 days and transmitted the disease in all trials. Kidney disease occurred among control fish in one of the trials, but in two others nearly 100 percent transmission was effected, and control lots were not found to have the disease during a reasonable period of observation. As an aside, Rucker et al. (1953) stated, "Also, we have not transmitted kidney disease by contact or feeding experiments." Snieszko and Griffin (1955) did not find kidney disease experimentally transmitted to eastern brook trout by 3 weeks of contact with infected fish followed by a single feeding of infected tissues. The differences which were observed may be due to the fact that different species of fish and different strains of the bacterium were used.

Because the method of feeding and contact did not transmit kidney disease to brook trout, the severe and highly artificial method of parenteral injection was used to provide fish for experimental chemotherapy.

Materials and Methods

Hatchery-raised eastern brook trout, averaging 42 grams at the start, were used for this experiment. They were fed a modified Cortland No. 6 diet (Tunison et al. 1939) at the rate recommended by Deuel et al. (1952) except on weekends when, for convenience, the quantity was halved. Water temperature was 12.5° C. Fish were graded for uniformity and were distributed in aliquots of 5 for a total of 25 fish for each trough. Two troughs of fish were used for each method of attempted transmission with each of the two strains of the bacterium.

The western strain of the bacterium was a laboratory culture which had been isolated from chinook salmon by Dr. E. J. Ordal and was labeled by him Butte Falls No. 110. The eastern strain of the bacterium was obtained from eastern brook trout at the U. S. Fish-Cultural station at Berlin, N. H. It was propagated by Dr. S. F. Snieszko in susceptible fish at Leetown, W. Va., for several years and was isolated on a culture medium for the first time shortly before this experiment was initiated.

Ordal and Earp's cysteine bloodagar (Ordal and Earp, 1956) was used as the culture medium for much of the work, but we have found that commercial Mueller Hinton medium (Difco)[1/] supplemented with 0.1 percent l-cysteine hydrochloride worked equally well, and some cultures were cultivated on it.

Control fish and experimental fish were maintained under identical conditions except that the control fish were not exposed to the bacterium. Half of the experimental fish were inoculated with the eastern strain of the bacterium; the remaining fish were inoculated with the western strain of the bacterium.

Five methods of attempted transmission were used. (1) The bacterium was added to the food of one group of fish. A moderately heavy growth from a 1- to 2-week-old slant culture was added to each 2 days' ration. (2) A second group also received the bacterium, but in addition their food included 5 percent glass shards which would pass through a 5-mm. screen but be retained by a 1.27-mm. screen. They were intended to scarify the gastro-intestinal tract and thus facilitate bacterial penetration. (3) Other troughs of fish received the specific bacterium plus 1 percent Difco No. 3 bile salts. Among the higher vertebrates, bile salts inhibit gastric HCl secretion and increase intestinal permeability. This, it was postulated, would favor survival of and penetration by the bacterium. (4) Rough bricks were placed in the troughs of another group and were rearranged each day. Water levels were lowered once each day, and the bacterium was added to these troughs at the low level. Fish were allowed to struggle half exposed to the air for 5 minutes after which the troughs were refilled. This treatment was intended to produce nose and abdominal abrasions like those often encountered among fish in concrete structures. (5) The last group of fish was manually abraded with 100-grit waterproof garnet abrasive paper for a similar reason.

1/ Mueller Hinton medium from other sources gave significantly less growth.

The abrasive was dipped into a suspension of the bacterium before each fish was scarified. Three different sites were abraded during the first week. Two additional sites were abraded during the second week, but because of fear of mortality from trauma the severity was reduced the next 3 weeks, and the earlier lesions were simply rubbed with bacterium-laden gauze. Even this appeared to be severe, and during the sixth to the ninth weeks inclusive, water was merely drawn down three times a week and charged with the bacterium. New abrasions were created in the 10th and 12th weeks, followed by mere addition of the bacterium to the lowered water during the final 3 weeks. These procedures were carried out for 102 days, after which some fish were held for additional observation.

By definition, kidney disease was considered to have been transmitted if the organism was presumptively identified by its morphology and staining characteristic in gram-stained smears of kidney or liver. It was considered to be the cause of death only if many cells of the bacterium were observed in most fields viewed under oil immersion. After the first mortality judged to be due to kidney disease, sample fish were periodically taken from all troughs, killed, and examined by staining smears of kidney and liver.

Results

About 60 days after the experiment began, several fish in the two troughs receiving the eastern strain of the bacterium in their food had symptoms of an external fungus infection (probably Saprolegnia sp.). All troughs of fish were treated with malachite green for 4 days, and the fungus infection was controlled. This was the only known occurrence of a confounding infection.

Kidney disease was not found in any of the control fish, and there was good agreement in the results obtained from the replicates.

No methods of feeding achieved transmission, and no mortality was caused by the feeding of bile salts or glass shards (table 1).

The eastern strain proved to be the more virulent and was readily transmitted by abrasion. It caused the first mortality among manually abraded fish at 48 days. All fish so treated were dead by the 102d day. Most of them died of kidney disease, but some may have died primarily from trauma. Fish subjected to conditions of self-abrasion did not begin to die until the 96th day, and the mortality continued at a low rate until the 288th day. Fish which remained were killed and examined but the kidney disease bacterium was not found.

The western strain established but few infections as determined by the methods used, and in only one fish was it considered to be the cause of death. Of the fish subjected to self-abrasion, only three died; one on the 211th day and two on the 296th day. The bacterium was also identified in one of the fish sampled during the 102-day period. One trough of the group manually abraded with the western strain was lost due to an accident on the 68th day, but the bacterium was presumptively identified in only one fish. Most of the fish in the other trough which had been manually abraded (15 fish) survived to the 296th day, but when killed, none was found to harbor the bacterium. All but the three mortalities in the self-abraded fish which remained after sampling survived to the 296th day: the kidney-disease bacterium was not identified in any of the fish killed at termination.

Discussion

Although none of the methods used resulted in establishing uniform infections in the groups on which it was tried, much useful information was obtained.

Clearcut differences were noted in the results obtained with the different strains of the bacterium. Because such factors as host species, length of time cultivation was carried out on artificial medium, and the like, there is at this time no importance implied to the geographic origin of these strains.

In strong contrast to ulcer disease and furunculosis, which are usually acute in eastern brook trout, the results of this experiment emphasize the chronic nature of kidney-disease

Table 1.--A comparison of results obtained from different methods of attempted transmission of fish kidney disease using two strains of the bacterium

	Fish killed for periodic examination		Fish killed for examination at termination date		Fish which died (all causes)	
	Infected with kidney disease	Healthy	Infected with kidney disease	Healthy	Infected with kidney disease	Other
Controls		18				1
Inoculated (50 fish each)						
Western Strain:						
Bacterium per os		18				
Bacterium and glass shards per os		18				
Bacterium and bile salts per os. .		18				
Self abrasion.	1	17	1	28	2	1
Manual abrasion.		12		15	1	22 1/
Eastern Strain: (50 fish each)						
Bacterium per os		18				
Bacterium and glass shards per os		18				
Bacterium and bile salts per os		18				
Self abrasion.		18		13	17	2
Manual abrasion.	6	9			26	8

1/ Accidental death

infection and point to the caution with which chemotherapy experiments must be designed in order not to terminate them prematurely.

Although the disease proved transmissible by abrasion, our observations of the response of the fish led us to believe that repeated inoculation was undoubtedly more effective than single inoculation in achieving this transmission. Even with the more effective abrasion (manual), the bacterium was not found in the kidneys of all the fish. This suggested either that infection did not occur or that it did but that recovery ensued. In any event, considering the fractional success and the time and effort involved, abrasion was judged to be less efficient than the severely artificial and highly fatal method of hypodermic injection.

The results of the experiment demonstrated that kidney disease was not transmitted to eastern brook trout by feeding. This corroborates the work of Snieszko and Griffin (1955) who did not find the disease transmitted to the same species of fish by feeding. In all, 300 fish, fish shown to be susceptible to infection by other routes, were fed the bacterium for a total of 102 days, and in no instance was transmission indicated. These are different results from those obtained among salmon under presumably different conditions on the West Coast. Admittedly there are great differences between the salmon and trout which are subject to kidney disease, but as-yet-unknown environmental factors may also be involved. As an example, kidney disease appears to be endemic in some trout-cultural stations in the eastern United States.

Although highly susceptible eastern brook trout are propagated in the facility operated in conjunction with our laboratory, there have been but 2 known occurrences of the disease, and in both instances fewer than 10 infected trout were found in circular pools in which thousands of trout were present.

In attempting to relate successful experimental infections achieved in conjunction with abrasion to hatchery occurrences among trout, several possibilities should be considered. The dermal route seems more likely to be involved in hatchery transmission than the oral route. In trout-cultural stations such dermal entry is theoretically possible following abrasion in masonry facilities or following biological penetration or lysis by any of several bacteria, fungi, or parasites to which these fish are hosts. An animal vector is not to be excluded as a possibility, but mere exposure of host tissue (mechanical or biological) is an equally attractive speculation.

EFFECTS OF TEMPERATURE ON KIDNEY DISEASE INFECTIONS

Mortality from kidney disease usually shows periodicity, but the more specific effects of temperature are less well established. Belding and Merrill (1935), the first to report on a disease which was apparently kidney disease, noted its seasonal occurrence and stated that the curve of mortality tended to follow the curve of rising water temperature. Earp et al. (1953) stated, "Water temperature is a major factor in determining the severity of the disease." They found that most outbreaks occurred during autumn and winter, the season of declining water temperatures, and that there was an increased mortality at the low temperatures. The most explosive outbreaks, however, were found to occur at warmer water temperatures. According to Snieszko and Griffin (1955), peak mortality at New Hampshire occurred during the spring - presumably when water temperatures were cold but rising. Wood and Wallis' data (1955) show salmon experiencing increasing mortality during periods of falling water temperatures, but they did not call particular attention to this. It was to determine more specifically the effect of temperature that the following experiment was conducted.

Material and Methods

Uniformly sized eastern brook trout averaging 66 grams each at the start were used for this experiment. Diet and rate of feeding were the same as in the preceding experiment. One unit of 2 troughs was kept at 7° C.; a second unit of 2 troughs was kept at 12.5° C. Water flow was adjusted within the limits imposed by the necessity of having to refrigerate one pair of troughs. Troughs at 12.5° C. received 900 ml. per minute; those at 7° C. received about 600 ml. per minute. Resulting average ammonia levels as periodically determined by nesslerization were about 0.8 p.p.m. at 7° C. and 0.4 p.p.m. at 12.5° C.

Ten fish from each trough were inoculated with the western strain, and ten were inoculated with the eastern strain. Inoculations were made at weekly intervals for the first 2 months and consisted of dual punctures made to a depth of 4 mm. with roughened needles dipped into a heavy suspension of the specific bacterium. Stable temperatures were maintained for 17 weeks, but with warm weather the capacity of the refrigeration unit was exceeded, and daytime temperatures rose several degrees during a period of 5 weeks. At that time continuation of refrigeration was deemed impractical, and the replicates were combined and moved to stable 12.5° C. water.

Results

There was good agreement in the results among the replicates used in this work. Of the two strains of the bacterium used, the eastern strain again proved to be the more virulent. The pattern of mortality for this strain was similar for both temperatures (fig. 1). At both 7° C. and 12.5° C. the peak mortality occurred about 90 days after the first inoculation. All fish of the 7° C. group were dead at the end of 170 days. Mortality continued about 60 days longer at 12.5° C., but only three fish were involved, and in the end all died.

At 12.5° C. the fish inoculated with western strain began to die about 60 days after the first mortality among those inoculated with eastern strain. As indicated by the slope of the line (fig. 1) the rate of mortality was low, and more than half the fish survived till termination at 302 days. They were killed and examined at that time. Only one fish had kidney disease; it had a surface lesion in which was found the kidney disease bacterium, but the organism could not be identified in smears made of the kidney. Mortality at 7° C. among fish inoculated with western strain began about 4 months after the first inoculation and 70 days after mortality began in fish inoculated with eastern strain. It reached a peak soon afterward, then gradually subsided. Four fish survived to the 302d day, and the causative organism was found in the kidney of one of them.

The development of lesions at the site of inoculation reflected the relative virulence of the two strains of the bacterium. At 12.5° C. eastern strain consistently evoked larger lesions than western strain. Except for a short time early in the experiment when the reverse was true, eastern strain lesions at 7° C. were also the larger. All lesions showed external regression toward the end of the second month -- just before mortality began among fish inoculated with the eastern strain.

Discussion

The fact that some fish which had been repeatedly inoculated by a shallow dermal puncture were found to be without bacteria about 10 months after inoculations began and 8 months after inoculations ceased can be interpreted as either evidence of probable recovery in an instance when initial infection sites were small and rather localized, or of failure of systemic infection to occur.

Similarly, the clear separation of mortality from each of the two strains of the bacterium, in spite of the fact that the fish were mixed in common troughs, is significant. This indicates that cross-infection did not occur, and that the disease is relatively difficult to transmit.

CONCLUSIONS

1. In eastern brook trout under hatchery condition, the oral route is not likely to be the usual portal of entry of the kidney disease bacterium.

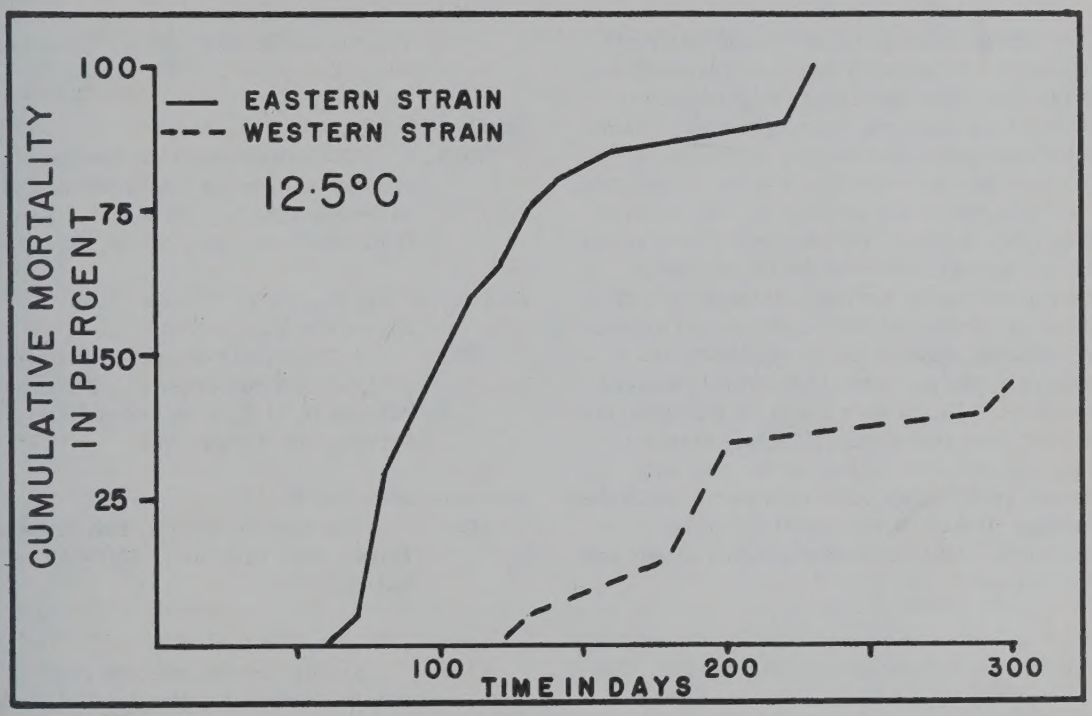
2. It is probable that the dermal route is the more likely portal of entry of this organism. Physical factors and/or biological agents which breach the skin barrier are highly suspect in initiating kidney disease infections in trout.

3. The methods of abrasion which were tried are considered incompletely effective and otherwise impractical for producing large numbers of uniformly infected fish.

4. Strains of the kidney disease bacterium can differ greatly in their ability to produce infection and disease.

5. Kidney disease is a slow-to-develop infection in eastern brook trout.

6. At two temperatures favorable for trout propagation, kidney disease produces greater losses at the lower temperature. The mechanism presumably involves a diminished host response.



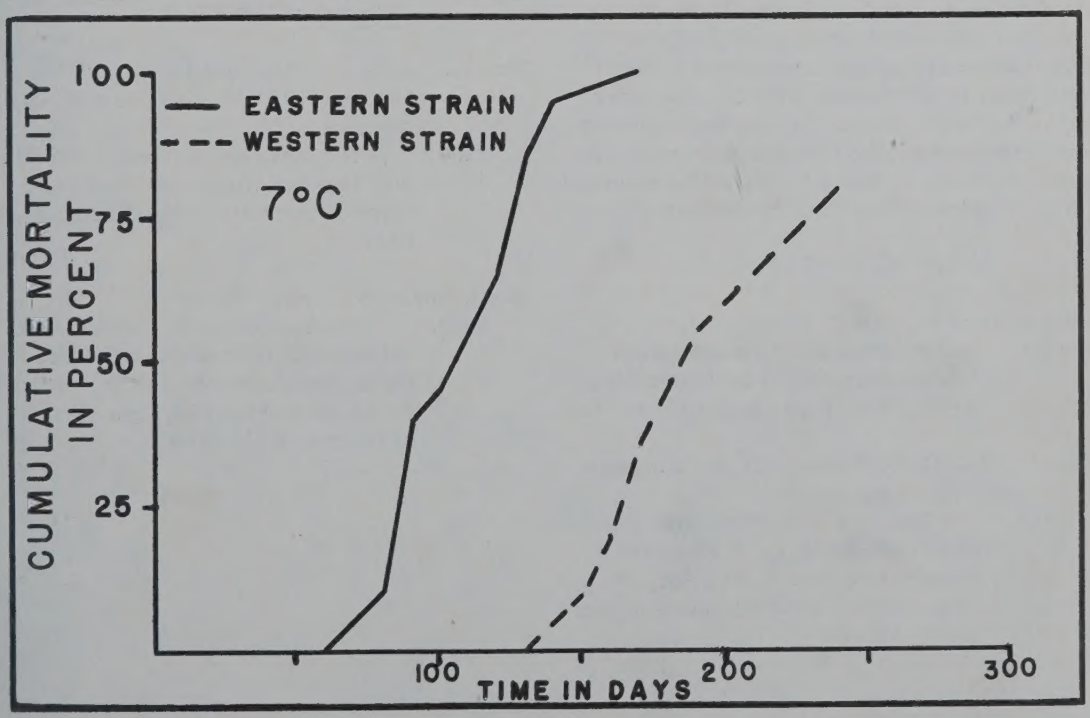


Figure 1:--Response at 7° C. and at 12.5° C. of eastern brook trout to repeated subdermal inoculations of an eastern and of a western strain of the fish kidney disease bacterium.

SUMMARY

In order to find a method of uniformly infecting large numbers of eastern brook trout, to determine how the disease might be transmitted in the hatchery, to compare two strains of the bacterium, and to observe the course of infection, transmission trials were carried out for 102 days in water at 12.5° C. Infection of eastern brook trout with kidney disease was not possible by addition of the bacterium to the fish's diet alone or with glass shards or with bile salts. Infection was accomplished by manual abrasion and to a lesser degree by auto-abrasion in the presence of the kidney disease bacterium. The eastern strain of the bacterium infected about one-third of the self-abraded group and about two-thirds of the manually abraded group. Few infections were established by either method with the western strain of the bacterium. All infections developed slowly and were chronic.

In an experiment intended to determine the effects of temperature on the disease, fish held at 7° C. and at 12.5° C. were given repeated puncture-type inoculations of the bacterium. One strain of the bacterium produced 100 percent mortality at both temperatures, but it was slightly more virulent at 7° C. The other strain was less virulent, but its effect was also more pronounced at the lower temperature. Recovery or failure to infect systemically occurred at both temperatures with this bacterium.

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